Genetically Divergent Strains of Feline Immunodeficiency Virus from the Domestic Cat (*Felis catus*) and the African Lion (*Panthera leo*) Share Usage of CD134 and CXCR4 as Entry Receptors[▽]

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The *env* open reading frames of African lion (*Panthera leo*) lentivirus (feline immunodeficiency virus [FIV$_{Ple}$]) subtypes B and E from geographically distinct regions of Africa suggest two distinct ancestries, with FIV$_{Ple}$-E sharing a common ancestor with the domestic cat (*Felis catus*) lentivirus (FIV$_{Fca}$). Here we demonstrate that FIV$_{Ple}$-E and FIV$_{Fca}$ share the use of CD134 (OX40) and CXCR4 as a primary receptor and coreceptor, respectively, and that both lion CD134 and CXCR4 are functional receptors for FIV$_{Ple}$-E. The shared usage of CD134 and CXCR4 by FIV$_{Fca}$ and FIV$_{Ple}$-E may have implications for in vivo cell tropism and the pathogenicity of the E subtype among free-ranging lion populations.

Lentiviruses are widespread pathogens of the Felidae, infecting both domestic and nondomestic felids (5, 6, 10, 21, 40, 41). In domestic cats (*Felis catus*), feline immunodeficiency virus (FIV$_{Fca}$) is a significant cause of disease, with infection resulting in a variable immunodeficiency syndrome characterized by recurrent gingivitis-stomatitis, cachexia, wasting, neuropathology, and an increased incidence of tumor development (1, 8, 9, 22, 26, 27, 37, 38, 52). The association between lentiviral infection and disease development in nondomestic felids is less certain, although African lions (*Panthera leo*) infected with the lion lentivirus FIV$_{Ple}$ show a dramatic decline in CD4$^+$ T lymphocytes (7, 30) and an expansion of an activated CD8$^+$ lymphocyte subpopulation expressing low levels of the CD8$\alpha\beta$ heterodimer, analogous to observations of FIV (35, 45) and human immunodeficiency virus (HIV) (33) infections.

FIV targets activated CD4$^+$ T lymphocytes by utilizing CD134 (OX40) as a primary attachment receptor (34) and CXCR4 as a coreceptor (43, 47, 51). Thus, receptor utilization is likely to be a significant contributory factor to the CD4$^+$ T lymphopenia observed in FIV-infected cats. Little is known about the receptor usage of the lentiviruses of nondomestic felids; however, the results of previous studies have indicated that the puma (*Puma concolor*) lentivirus FIV$_{Pco}$-1695 and subtype B lion lentivirus FIV$_{Ple}$-458 use alternative attachment receptors from FIV$_{Fca}$ (36). If neither the lion nor the puma lentiviruses use CD134 or CXCR4 as viral receptors, how then do they induce a depletion of CD4$^+$ lymphocytes?

Proviral genome sequence analysis of the subtype B and E lion lentiviruses indicated that the FIV$_{Ple}$-B *env* was most closely related to the virus of the Asiatic Pallas' cat (*Felis manul*) (4), whereas the FIV$_{Ple}$-E *env* was more similar to that of the domestic cat virus FIV$_{Fca}$ (25) than to FIV$_{Ple}$-B. These data raise the possibility of either an ancient recombinatorial event between FIV strains in the wild followed by a substantial period of divergence or a more-recent recombinatorial event with an as-yet-uncharacterized but highly divergent FIV species from lions or another lentivirus-infected African species. Here, we ask whether the phylogenetic relationship between FIV$_{Ple}$-E and the domestic cat virus FIV$_{Fca}$ is reflected in the receptor usage of the two viruses.

Peripheral blood mononuclear cells were isolated from 15 seropositive lions from the Moremi game reserve in the Okavango Delta region of Botswana, and one animal (Sangre) yielded replication-competent lentivirus. Sangre was an 8-year-old male lion in good health at the time of sampling. The FIV$_{Ple}$-E-Sangre *env* gene was cloned, and its predicted amino acid sequence displayed 87% identity with the FIV$_{Ple}$-1027 isolate of FIV$_{Ple}$-E (25) across the SU-TM coding sequence and 54% identity with FIV$_{Fca}$-GL8 (46). FIV$_{Ple}$-E and FIV$_{Fca}$ HIV(FIV) luciferase pseudotypes were prepared (34) and their receptor usages compared (Fig. 1A). Both FIV$_{Ple}$-E and FIV$_{Fca}$-GL8 infected cells expressing feline, but not human, CD134. Neither FIV$_{Ple}$-E nor FIV$_{Fca}$-GL8 utilized feline cysteine-rich domain 1 (CRD-1) against human CD134 (48, 50) chimera, suggesting that the FIV$_{Ple}$-E Env interacts with CD134 in a manner similar to FIV$_{Fca}$-GL8 (48).

Next, we cloned the lion homologues of CXCR4 and CD134 and expressed both molecules in human NP2 cells. Lion and feline CD134 were coexpressed with either feline or lion CXCR4 by retroviral transduction, and the cells were challenged with FIV$_{Fca}$ pseudotypes. Each of the four receptor/coreceptor combinations rendered the cells permissive for infection with HIV(FIV$_{Fca}$) pseudotypes bearing diverse Envs

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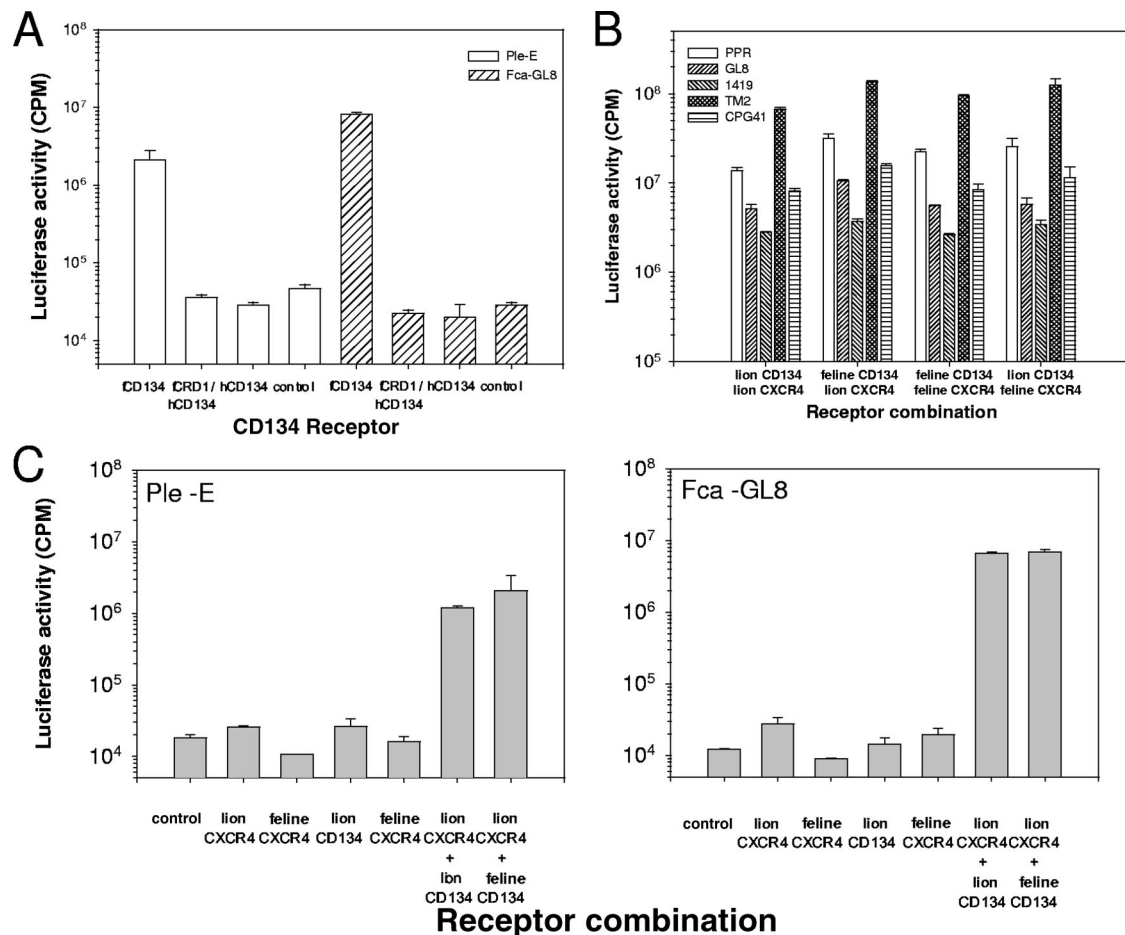
FIG. 1. Receptor utilization by FIV$_{Fca}$ and FIV$_{Ple}$-E. HIV-luciferase pseudotypes were prepared bearing the Envs of either FIV$_{Fca}$ or FIV$_{Ple}$-E and used to infect MCC cells stably transduced with retroviral vectors bearing feline (domestic cat) CD134 (fCD134), feline CD134 CRD1/human CD134 chimera (fCRD1/hCD134), human CD134 (hCD134), or vector only (control) (A); NP2 cells transduced with retroviral vectors bearing lion CD134, lion CXCR4, feline CD134, or feline CXCR4 in combination (B); or lion CD134, lion CXCR4, feline CD134, or feline CXCR4 alone or lion CXCR4 in combination with lion or feline CD134 (C). Cells were infected with pseudotypes bearing FIV$_{Ple}$-E or FIV$_{Fca}$-GL8 (A); FIV$_{Fca}$-PPR, GL8, 1419, TM2, or CPG41 (50) (B); or FIV$_{Ple}$-E or FIV$_{Fca}$-GL8 (C). Histograms are representative of the results of at least two independent experiments and display means ± standard errors (n = 3).

(Fig. 1B) with a similar efficiency, confirming that lion CD134 and CXCR4 were functional receptors for FIV$_{Fca}$. Stable NP2-derived lines were then generated expressing lion CD134, lion CXCR4, feline CD134, and feline CXCR4. The cells expressing lion CXCR4 were then transduced again with vectors carrying feline or lion CD134 and selected by immunomagnetic separation (MACS; Miltenyi Biosciences). The cells were then infected with FIV$_{Ple}$-E and FIV$_{Fca}$-GL8 pseudotypes, and viral entry was assessed (Fig. 1C). The coexpression of lion CXCR4 in conjunction with feline or lion CD134 (but not CXCR4 expression alone) rendered the cells permissive to infection with both FIV$_{Ple}$-E and FIV$_{Fca}$-GL8. Thus, FIV$_{Ple}$-E infection requires the coexpression of both CD134 and CXCR4, and lion CD134 and CXCR4 are functional primary receptors and co-receptors, respectively. Next, eukaryotic expression vectors bearing the FIV$_{Ple}$-E and FIV$_{Fca}$-GL8 Envs were transfected directly into AH927 cells stably expressing feline CXCR4 (42) (AH927-FX4P) and feline CD134. Transfection of either the Ple-E (Fig. 2B) or GL8 (Fig. 2D) env into AH927-FX4P-CD134 cells resulted in syncytium formation, while the results

for transfected AH927-FXP-Control cells (Fig. 2A and C, respectively) did not differ significantly from those for mock-transfected cells.

FIV$_{Fca}$ strains vary in their sensitivity to the inhibition of viral entry by soluble CD134L (49) and the CXCR4 antagonist AMD3100 (49). The sensitivity of FIV$_{Ple}$-E, FIV$_{Fca}$-GL8, and FIV$_{Fca}$-B2542 (14) to soluble feline CD134L and AMD3100 was assessed with MYA-1 (24) cells. AMD3100 inhibited infection with all three viral pseudotypes in a dose-dependent manner (Fig. 3A and C), with ≥95% inhibition of infection at 400 ng/ml antagonist. In contrast, while GL8 pseudotypes resisted inhibition by soluble CD134L at all but the highest concentration (50 μg/ml) (Fig. 3B and D), infection with the B2542 and FIV$_{Ple}$-E pseudotypes was markedly reduced at 0.4 μg/ml CD134L (~70% inhibition at 0.4 μg/ml) (Fig. 3D).

FIV replication in canine CLL cells is CD134 dependent (50); we therefore assessed the growth of FIV$_{Ple}$-E in CLL cells, asking whether CD134 expression was essential for viral growth. FIV$_{Ple}$-E was compared with FIV$_{Fca}$-GL8, a strain of FIV for which productive infection is CD134 dependent, and
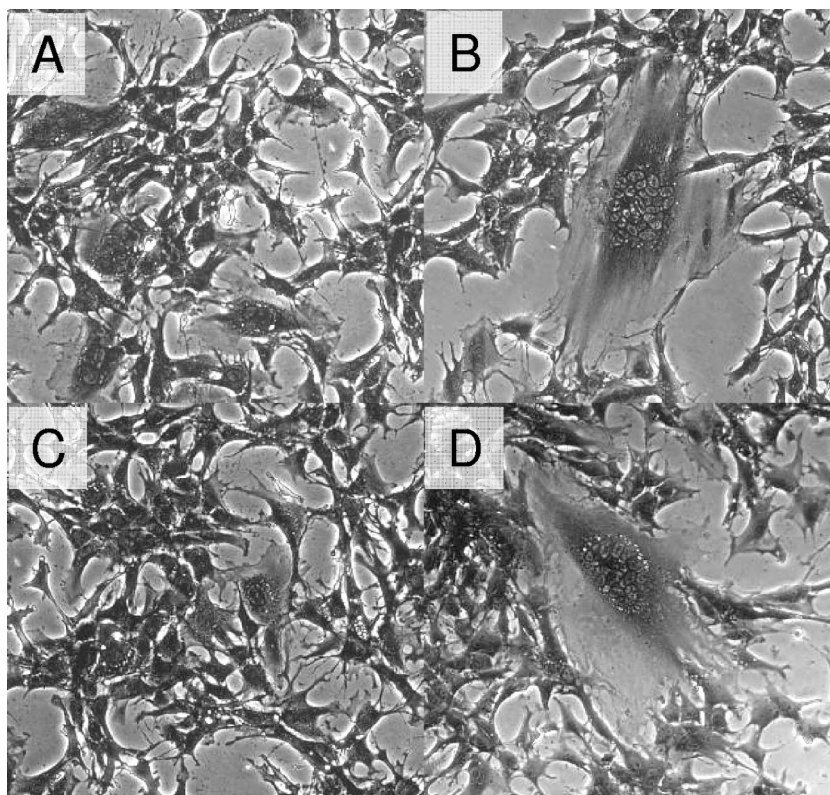
FIG. 2. Syncytium formation in FIV$_{Ple}$-E *env*-transfected cells. AH927 cells expressing feline CXCR4 (A, C) or feline CXCR4 plus feline CD134 (B, D) were transfected with eukaryotic expression vectors bearing FIV$_{Ple}$-E (A, B) or FIV$_{Fca}$-GL8 (C, D) *env*. Monolayers were fixed and stained at 48 h posttransfection, and photographed representative images are shown.

FIV$_{Ple}$-458 (6), a subtype of FIV$_{Ple}$ known to infect independently of CD134 and CXCR4 expression (36, 44). CD134 expression rendered CLL cells permissive for productive infection with FIV$_{Fca}$-GL8 and FIV$_{Ple}$-E, while control cells were resistant to infection with either virus (Fig. 4). In contrast, both CLL and CLL-CD134 were susceptible to infection with FIV$_{Ple}$-458, confirming that infection with FIV$_{Ple}$-458 is CD134 independent.

In this study, we demonstrate that FIV$_{Ple}$-E-Sangre shares a common receptor and coreceptor with the lentivirus of the domestic cat. There have been conflicting reports regarding the pathogenicity of lentiviruses in nondomestic felids, and the observation that two distinct biological phenotypes can be discerned between subtypes B and E of FIV$_{Ple}$ may indicate distinct pathogenicities in vivo. The virus-receptor interaction is a critical determinant of viral cell tropism and cytopathicity, and FIV$_{Fca}$ targets activated CD4$^+$ T cells primarily due to the restricted expression of the primary receptor CD134 and coreceptor CXCR4 in this T-cell compartment (12, 34, 49). Accordingly, a decline in circulating CD4$^+$ T lymphocytes is associated with FIV infection of the domestic cat (1). The shared usage of CD134 and CXCR4 between FIV$_{Fca}$ and FIV$_{Ple}$-E would be consistent with a decline in CD4$^+$ T lymphocytes following FIV$_{Ple}$-E infection of lions. Previous studies have observed that both captive (7) and free-ranging (7, 30) lion populations show depletions of CD4$^+$ T lymphocytes consistent with selective targeting of this T-cell subset by the virus. A study of free-ranging lions (30) examined blood samples from

animals in Tanzania and South Africa, where FIV$_{Ple}$ subtypes A, B, and C have been described previously (6). As FIV$_{Ple}$-B infection appears to be independent of CD134 expression, future studies should address whether usage of CD134 as receptor by FIV$_{Ple}$-E facilitates more efficient targeting of activated CD4$^+$ T lymphocytes and a more marked immunopathology among free-ranging lions in countries such as Botswana, where the subtype E virus has been described (41). Similarly, FIV$_{Ple}$-B may have acquired usage of a distinct cell surface molecule that is expressed on CD4$^+$ T lymphocytes as a means of targeting this subpopulation. Alternatively, CD4$^+$ lymphocyte targeting may not solely be the result of restricted receptor expression; rather, it may be the sum of a sequence of events: the trapping of virions on dendritic cells in the lymph node through DC-SIGN interactions (HIV, simian immunodeficiency virus, and FIV$_{Fca}$) (3, 13, 18–20, 28, 29), an interaction with a primary receptor found on CD4$^+$ T cells (CD4 for HIV/simian immunodeficiency virus [11, 23, 32] and CD134 for FIV [34]), and a subsequent interaction with seven-transmembrane domain family molecules, such as CCR5 or CXCR4 (2, 15–17, 31, 39, 47, 51). Thus, in order to target CD4$^+$ T cells, the primary receptor for the virus may not necessarily be restricted in expression to CD4$^+$ T cells; specificity may result from restricted expression of the viral coreceptor or from selective trafficking of CD4$^+$ T cells through the dendritic-cell-rich regions of lymphoid tissues where virions are trapped. Comparative studies between the felid and the primate lentiviruses will go some way to addressing the relative significance
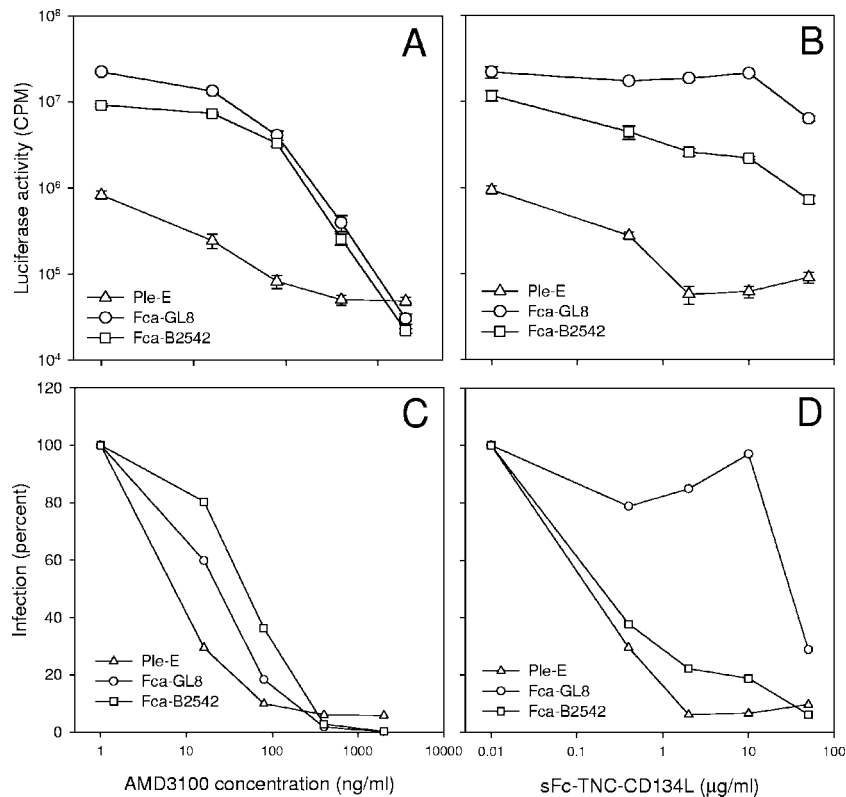
FIG. 3. Sensitivity of FIV$_{Ple}$-E to AMD3100 and soluble CD134L. MYA-1 T cells were infected with HIV(FIV)-luciferase pseudotypes bearing Envs from FIV$_{Ple}$-E or FIV$_{Fca}$-GL8 and B2542 in the presence of increasing concentrations of CXCR4 antagonist AMD3100 (A, C) or soluble Fc-TNC-CD134L (sFc-TNC-CD134L) (B, D). (A, B) Luciferase activity at 72 h postinfection. Each point represents the mean ($n = 3$) $\pm$ standard error. CPM, counts per minute. (C, D) Percent infection relative to mean infectivity of control with no antagonist. TNC, tenascin.

of the virus-receptor interaction to the depletion of CD4$^+$ T lymphocytes.

FIV$_{Fca}$ strains vary in their sensitivity to antagonism by soluble CD134L (49). When infection with FIV$_{Ple}$-E was compared with that of FIV$_{Fca}$ strains GL8 (46) and B2542 (14), FIV$_{Ple}$-E infection was found to be sensitive to modulation by CD134L, suggesting that the interaction between FIV$_{Ple}$-E Env and CD134 is of similar affinity to that of B2542. However, unlike B2542, FIV$_{Ple}$-E Env-bearing pseudotypes required de-

terminants in CRD-1 and -2 of CD134 for infection, similar to GL8 and CPG41 (50). The nature of the interaction between Env and CD134 may correlate with the propensity of the virus to interact directly with CXCR4 (reviewed in reference 44). FIV$_{Ple}$-E is the first virus we have identified to date that is sensitive to CD134L and yet interacts with CD134 in a manner similar to "early" isolates such as GL8 and CPG41, indicating that the two properties are not mutually exclusive. Future studies should investigate whether endogenous lion CD134L
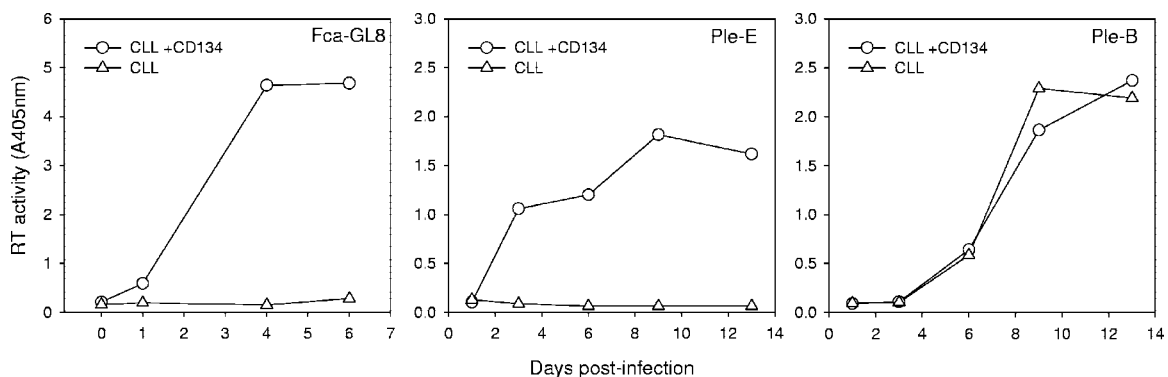

FIG. 4. Productive infection with FIV$_{Ple}$-E is CD134 dependent. Canine CLL cells (CLL) or CLL cells stably transduced with retroviral vectors bearing feline (domestic cat) CD134 or vector only (CLL+CD134) were infected with MYA-1 T-cell-grown FIV$_{Fca}$-GL8, FIV$_{Ple}$-E, or FIV$_{Ple}$-B, and viral replication was quantified by nonisotopic reverse transcriptase (RT) assay (colorimetric assay; absorbance at 405 nm).

plays a role in controlling viral replication and ameliorating viral pathogenicity.

FIV$_{Ple}$-E and FIV$_{Ple}$-B differ in their capacities to replicate in feline cell lines (36, 44), their sensitivity to receptor antagonists (36, 44), and their requirement for ectopic expression of CD134 for productive infection. Here, we provide further compelling evidence that FIV$_{Ple}$-B utilizes a distinct receptor(s) for infection, replicating efficiently in CD134-negative cells. If the differences in receptor usage between FIV$_{Ple}$-E and FIV$_{Ple}$-B affect cell tropism and pathogenicity in vivo, this will be of significance to the management of both free-ranging endangered felids and breeding populations in captivity.

Nucleotide sequence accession numbers. The sequences of lion CD134, CXCR4, and FIV$_{Ple}$-E-Sangre have been deposited in GenBank under accession numbers EU915482, EU915483, and EU915484.

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